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Salivary flow rate and pH in patients with oral pathologies

P.L. FOGLIO-BONDA[1], K. BRILLI[2], F. PATTARINO[3], A. FOGLIO-BONDA[3]

[1]Department of Translational Medicine, University of Eastern Piedmont, Novara, Italy
[2]Dental Clinic, University of Eastern Piedmont, Novara, Italy
[3]Department of Pharmaceutical Science, University of Eastern Piedmont, Novara, Italy

Abstract. – OBJECTIVE: Determine salivary pH and flow rate (FR) in a sample of 164 patients who came to Oral Pathology ambulatory, 84 suffering from oral lesions and 80 without oral lesions. Another aim was to evaluate factors that influence salivary flow rate.

PATIENTS AND METHODS: Subjects underwent clinical examination and completed an anamnestic questionnaire in order to obtain useful information that was used to classify participants in different groups. Unstimulated whole saliva (UWS) was collected using the spitting method at 11:00 am. The FR was evaluated with the weighing technique and a portable pHmeter, equipped with a microelectrode, was used to measure pH. Both univariate and classification (single and Random Forest) analyses were performed.

RESULTS: The data analysis showed that FR and pH showed significant differences ($p < 0.001$) between patients with oral lesions (FR = 0.336 mL/min, pH = 6.69) and the ones without oral lesions (FR = 0.492 mL/min, pH = 6.96). By Random Forest, oral lesions and antihypertensive drugs were ranked in the top two among the evaluated variables to discretize subjects with FR = 0.16 mL/min.

CONCLUSIONS: Our study shows that there is a relationship between oral lesions, antihypertensive drugs and alteration of pH and FR.

Key Words:
Human saliva, Salivary pH, Salivary flow rate, Oral diseases.

Introduction

Saliva is a remarkably complex fluid with a large number of properties and functions which are indispensable for both general and oral health like lubrication, moistening, taste, digestion, protection of the oral and esophagus mucosa and tooth protection[1]. The salivary flow rate (SFR) is the amount of saliva produced by salivary glands in the time unit, expressed in mL/min or g/min. It can be divided into unstimulated (USFR) which is independent of the presence of stimuli (food, chewing, etc.) and stimulated (SSFR), secreted in response to sensory stimulation, gustatory and masticatory mainly[2,3]. Moreover, saliva can be divided into "Duct saliva" that is the fluid obtained immediately downstream of the ducts of the salivary glands and "Whole saliva", the fluid composed by "Duct saliva" with the addition of the secretions of oral, nasal and pharynx mucosa; this fluid also contains microorganisms, desquamated epithelial cells, blood cells, food debris, e.g.[4,5].

Saliva chemical and physical properties play an important role in maintaining the health and functions of the oral cavity. Lubrication of alimentary bolus, protection against virus, bacteria, and fungi, buffer capacity, protection and reparation of oral mucosa and dental remineralization are some of the functions of saliva[3,4,6]. The buffer capacity depends on the acids and bases contained in the secreted saliva[7,8]. Bicarbonate is the main buffer that opposes acids, but is completely effective only at high salivary flow rates, because its concentration increases markedly with SFR rise[8,9]. It's well known that patients with quantitative and/or qualitative alterations in saliva may complain about oral dryness sensation, suffering from difficulties in eating, speaking and swallowing; furthermore, in these altered conditions, dental caries, opportunistic infections, and diseases of the oral cavity may increase[2-6,10-14]. About 20% of the general population suffer from dry mouth[11,13,15].

The evaluation of the unstimulated whole salivary flow rate (UWSFR), is carried out by an easy, non-invasive and comfortable procedure, which favors its use in clinical environmental (public or private). UWSFR is the basal rate of saliva flow and it's the greatest contributor to the total salivary

Corresponding Author: Pier Luigi Foglio-Bonda, MD; e-mail: pierluigi.fogliobonda@unipmn.it

output during the diurnal cycle[16,17]. The collection of unstimulated "whole saliva" reflects basal SFR; this fluid is present in our mouths for about 14 hours a day and its secretion provides protection to oral tissues[6]. SSFR represents the secretion during food intake, and occur in our mouths for up to 2 hours[6,18]. Furthermore, stimulating the flow of saliva can alter its composition; for example, the concentration of bicarbonate which increases progressively with the duration of stimulation[16,17]. What above leads us to consider UWSFR as a more clinically reliable parameter.

There are conflicting data in literature concerning SFR[6,8,10,11,19-21] and pH[6,8,14,22-24] in UWSFR and publications about their correlation[14,25]. The effects of physiological factors such as gender, body profile, salivary gland size and bite force on the SFR and pH of saliva are controversial[11,14,19,26]. Moreover, several pathological and behavioral factors could influence UWSFR: e.g. oral and systemic diseases, drugs intake, nutrition, stress, sports activity[1-5,10-13,15,17,27].

The aims of our observational prospective study were: to determine the UWSFR and corresponding pH in a sample of 164 patients; describe the donor evaluating the following variables: gender, age, smoking (nicotine dependency), drinking alcohol, oral lesions, drug recognized as anxiolytic, systemic diseases like diabetes mellitus, arterial hypertension, gastroesophageal diseases, hepatitis, neoplastic and heart diseases; investigate how UWSFR could be described by these indicators. In order to reduce UWSFR variability, a rigid protocol to enroll the donor cohort and strict behavioral norms to control subjects during sampling were applied[2,3,6,7].

Patients and Methods

The study involved 164 subjects (95 females and 69 males with a mean age of 56.88 years old, SD 12.39) recruited from Oral Pathology Clinic ambulatory of AOU Maggiore della Carità di Novara. All subjects were selected in a homogeneous (Caucasian) population. They were informed of the purpose of the study, approved by the local Ethics Committee (N. RQ3010), and enrolled after giving their signed informed consent. All subjects answered an anamnestic questionnaire acknowledging systemic diseases: arterial hypertension, diabetes mellitus, gastroesophageal diseases, hepatitis, neoplastic diseases, heart diseases and harmful habits (smoking-alcohol).

An identification code consisting of a letter and a number was assigned to each subject, who was submitted to an oral examination during which particular attention was given to the condition of the mucous membrane. The oral examination was performed by a medical doctor, expert in oral medicine and trained in salivary testing.

The enrolled subjects were submitted to a rigid protocol of behavioral norms: in the two previous weeks, they had to avoid consumption of chewing gum; the day before saliva collection, they had to be relaxed and not to practice sports activity. In the sampling day participants had to be free from symptom of fever and/or cold; if they were hungry or thirsty, they could eat or drink water, but later immediately they had to clean their teeth with a provided toothpaste; during the last hour before the salivary collection, it was not permitted them to eat, to drink or to smoke.

All subjects were experienced, during the test, in the Province of Novara (Italy) or surrounding areas.

The UWSFR was detected under controlled temperature (22-24°C) and humidity conditions (75% ± 5%) at 11:00, using the spitting method[2,26]. Unstimulated whole saliva (UWS) was collected for a 5 min time span: the undisturbed subject, sitting in a comfortable position, swallowed residual saliva present in the mouth before the beginning of the collection and then, with the head down and mouth slightly open, saliva was allowed to drip from the lower lip into a pre-weighed, sterile plastic test tube. In the last few seconds of the 5 min, saliva accumulated in the mouth was spat out into the plastic funnel. No other conscious movements of the oral musculature were made during the collection.

The salivary samples were weighed using a Precisa Balance, Series Bj (Dietikon, Zurich, Switzerland) to determine the UWSFR, which was calculated by dividing the net weight of saliva by the five minutes of the collection period.

The UWSFR was reported as g/min, which is nearly equivalent to mL/min; preliminary studies revealed that weight and volume of UWS were very highly correlated, but that volume measures were less reliable[2,4,10,28].

pH was immediately detected on samples by a portable pH meter (HI 9026, Hanna Instruments, Burlington, Vermont, NE, USA) equipped with a special 5 mm diameter electrode.

Data from anamnestic questionnaires and clinical examination were computed to classify different variables. Variable "Alcohol Yes" was defined

if subject drank wine, beer or super-alcoholics reaching a value of 210 mL of Ethanol for week[29]. Variable "Anxiolytics" with level "Yes" was defined to those subjects who regularly take (one assumption/day) any drug recognized as anxiolytic. Variable pH was organized in two levels: subjects with salivary pH ≥ 6.5 were named "Normal", while, if salivary pH was lower that 6.5, subjects belong to "Low" group[29]. UWSFR variable was organized by tertile classification of measured UWSFR resulting the following groups: Low = 0.046-0.264 mL/min, Medium = 0.265-0.451 mL/min and High = 0.451-1.850 mL/min. Variable "Smoke" with level "Yes" was defined to those subjects who smoke more than 2 times/day. Variable "Prosthesis" was organized in two levels for univariate analysis (No or Yes), while for classification analyses it was organized in three levels: subjects were divided into three levels "Prosthesis", "Skeletal" and "No" corresponding to subjects who wore respectively dental removable prosthesis, dental skeletal prosthesis or did not have any oral prosthesis. Variables "Diabetes mellitus", "Hypertension", "Gastric diseases", "Heart diseases", "Hepato" and "Cancer" with level "Yes" were defined to those subjects who suffer from corresponding pathologies. Variable "Lesion" was organized in two levels for univariate analysis (No or Yes), while for classification analyses in three levels: subjects with a diagnosis of Lichen Planus were named "Lichen", those who suffer from Leukoplakia belong to "Leuko" group. Subjects without any of these pathologies were named "No". "Healthy teeth" and "Age" variables were used as continuous ones.

Statistical Analysis

Univariate Analysis

The variables were descriptively analyzed with mean, maximum, minimum, standard deviation (SD), including their relative standard deviation (RSD%). Univariate analyses were performed, using the Student's *t*-test or Wilcoxon rank sum test if data were not normally distributed, on pH and UWSFR data organized in groups according to each single variable as previously described.

Classification Analysis

UWSFR was used as response variable organized as previously described while other variables as descriptive ones (ranking). Fisher's Exact Test or Kruskal-Wallis rank sum test were performed for each descriptive variable depending if the variable was categorical or continuous type respectively. Random Forest analysis on response variable was repeated for several training/test (0.6/0.4) splits and models were evaluated with confusion matrix and receiver operating characteristic curve (ROC). The selection of descriptive variables started using all of them and then by removing the ones highly correlated or those having poor cases for an ideal training/test division. The data analysis was carried out with procedures implemented by R software 3.1.0; a p-value of ≤ 0.05 was considered statistically significant in all of the tests.

Results

The classification analysis carried out on the population divided into UWSFR groups (Low, Medium, High) did not show significant results both in single and in Random Forest analyses (data not showed). In order to select a better organization for response variable, UWSFR values were organized following the method proposed by Fenoll-Palomares et al[6] (Pathological ≤ 0.16 mL/min and Normal >0.16 mL/min) (Table I). This classification was used also in a univariate analysis performed on pH values organized according to UWSFR groups.

Mean UWSFR of all 164 patients was 0.405 ml/min (SD = 0.277; RSD = 68.43%). The mean UWSFR of 88 patients with oral lesion was 0.35 mL/min (SD = 0.29; RSD = 84.06%). The mean UWSFR value of 80 patients without mucosal oral lesions was 0.492 ml/min (SD = 0.25; RSD = 51.06%). This difference (0.156 mL/min) was statistically significant; moreover, some other variables showed to significantly affect the salivary flow rate (Table I).

Mean pH of all the 164 patients was 6.82 (SD = 0.35; RSD = 5.19%). The mean pH of 88 patients with oral lesion was 6.70 (SD = 0.38; RSD = 5.77%). The mean pH of 80 patients without mucosal oral lesions was 6.95 (SD = 0.23; RSD = 3.37%). This difference (0.26) was statistically significant; furthermore, the response resulted significantly related to smoke variable (Table I).

The results obtained from classification analyses using population divided in UWSFR two groups (Normal, Pathological) are showed in Table I. Results similar to those obtained from the univariate analyses were observed, nevertheless some variables have lost significance (Hepato, Cancer). The Random Forest model ranked the

Table I. Donors' population features from univariate and classification analyses.

	Univariate analyses Significance test (p)		Classification analyses			
Variables	UWSFR	pH	Levels	UWSFR group pato (n= 23)	UWSFR group normal (n= 141)	Significance test (p)
Gender	0.748	0.071	Male	9	60	0.997
			Female	14	81	
Age	-	-	Continuous	-	-	0.308
Smoking	0.311	0.035	Yes	4	44	0.222
			No	19	97	
Prosthesis	0.518	0.849	No	19	111	0.843
			Prosthesis	3	17	
			Scheletric	1	13	
Alcohol	0.801	0.875	Yes	2	13	0.997
			No	21	128	
Hypertensive diseases	<0.001	<0.001	Yes	6	11	0.002
			No	17	130	
Anxiolytic drugs	0.247	0.756	Yes	0	6	0.596
			No	23	135	
Healthy teeth	-	-	Continuous	-	-	0.734
Diabetes mellitus	0.094	0.773	Yes	1	2	0.366
			No	22	139	
Gastric diseases	0.175	0.415	Yes	3	8	0.186
			No	20	133	
Heart diseases	0.126	0.525	Yes	4	9	0.088
			No	19	132	
Hepatology	0.007	0.763	Yes	2	1	0.052
			No	21	140	
Cancer	0.022	0.536	Yes	3	4	0.058
			No	20	137	
Lesion	<0.001	<0.001	No	2	78	<0.001
			Lichen	14	39	
			Leuco	7	24	
pH	0.023	-	Normal	18	127	0.150
			Low	5	14	
UWSFR normal/pato groups	-	0.125	-	-	-	-

first 5 variables as follow: Lesion, Hypertension, Anxiolytics, Gastric diseases, Heart diseases. Mean Area Under curve in ROC test was 0.91 on training and 0.69 in the testing set.

Discussion

Univariate analysis showed that the presence of lichen and leukoplakia in oral cavity reduces the UWSFR values. This finding is in agreement with Bergdhal et al[11], that showed how women with oral lesion complaints had a lower unstimulated salivary flow.

Also in classification analyses, oral lesion plays an important role in discretizing UWSFR "Pathological" group from the UWSFR "Normal" one. Unfortunately, due to a limited number of cases, several variables, which were significant in univariate analyses, were removed in classification analyses. On the other hand, the "Hypertensive Diseases" variable was found significant both in univariate and in classification analyses (second ranked). These results are in agreement with several papers where interaction was found between Antihypertensive drugs intake and reduced salivary flow rate (Bergdhal et al[11], Nagler et al[27]). Moreover, a significant alteration of pH in Hypertensive patients was also detected in univariate analyses; in this case, it is to be expected that such a decrease in pH is related to the impact caused by antihypertensive drugs on UWSFR. The variables Hepatopathies and Cancer were not significant for UWSFR in classification analysis. Since the

number of patients suffering from these pathologies was limited, probably this classification is not effective and for this reason, Hepatopathies and Cancer variables have been excluded from the Random Forest analysis. We may suppose an influence of these pathologies on homeostasis of the oral cavity, as already suggested by Torres et al[30], but additional studies targeted to these two diseases will be required.

Conclusions

From the results of our research is inferable that different factors can influence the UWSFR especially hypertension; in fact this variable can detect possible "risk patients" with altered oral homeostasis. Other variables such as cancer or liver disease may be an additional risk factor for the decrease of the UWSFR with repercussions on homeostasis of the oral cavity. It would be desirable, in our opinion, that all professionals who deal with oral health pay attention to patients under antihypertensive drugs treatment, subjects with liver diseases and cancer, especially in old age.

Consent

Written informed consent was obtained from all of the subjects.

Ethical approval

The study was approved by our Faculty Ethics Committee n° RQ3210.

Conflict of Interest

The authors declare that they have no competing or conflicting interests. Each author certifies that he or she has no commercial associations that might pose a conflict of interest connection with the submitted article. No funding sources supported this work.

References

1) Dawes C, Pedersen AM, Villa A, Ekström J, Proctor GB, Vissink A, Aframian D, McGowan R, Aliko A, Narayana N, Sia YW, Joshi RK, Jensen SB, Kerr AR, Wolff A. The functions of human saliva: a review sponsored by the world workshop on oral medicine VI. Arch Oral Biol 2015; 60: 863-874.
2) Foglio Bonda PL, Migliario M, Rocchetti V, Pattarino F, Foglio Bonda A. Daily and annually variation of unstimulated whole saliva flow rate and pH and their relation with body profile in healthy young adults. Eur Rev Med Pharmacol Sci 2013; 17: 253.
3) Edgar WM. Saliva: its secretion, composition and functions. Br Dent J 1992; 172: 305-312.
4) Navazesh M, Christensen CM. A comparison of whole mouth resting and stimulated salivary measurement procedures. J Dent Res 1982; 61: 1158-1162.
5) Mandel ID. A contemporary view of salivary research. Crit Rev Oral Biol Med 1993; 4: 599-604.
6) Fenoll-Palomares C, Munoz-Montagud JV, Sanchiz V, Herreros B, Hernandez V, Minguez M, Benages A. Unstimulated salivary flow rate, pH and buffer capacity of saliva in healthy volunteers. Rev Esp Enferm Dig 2004; 96: 773-783.
7) Moritsuka M, Kitasao Y, Burrow MF, Ikeda M, Tagami J. The pH change after HCL titration into resting and stimulated saliva for a buffering capacity test. Aust Dent J 2006; 51: 170-174.
8) Wang P, Zhou Y, Zhu YH, Lin HC. Unstimulated and stimulated salivary characteristics of 12-13 year-old schoolchildren with and without dental erosion. Arch Oral Biol 2011; 56: 1328-1332.
9) Kteusser W, Heidland A, Henneman H, Wigand ME, Knauf H. Mono- and bivalent electrolyte patterns pCO2 and pH in relation to flow rate in normal human parotid saliva. Eur Clin Invest 1999; 44: 398-406.
10) Navazesh M, Mulligan RA, Kipnis V, Denny PA, Denny PC. Comparison of whole saliva flow rates and mucin concentrations in healthy Caucasian young and aged adults. J Dent Res 1992; 71: 1275-1278.
11) Bergdahl M. Salivary flow and oral complaints in adult dental patient. Community Dent Oral Epidemiol 2000; 28: 59-66.
12) Chaushu G, Bercovici M, Dori S, Waller A, Taicher S, Kronenberg J, Talmi YP. Salivary flow and its relation with oral symptoms in terminally ill patients. Cancer 2000; 88: 984-987.
13) Sreebny LM. Saliva in health and disease: an appraisal and update. Int Dent J 2000; 50: 140-161.
14) Wu PK, Ke JY, Chung CY, Chen CL, Hwang TL, Chou1 MY, Wong MKA, Hu CF, Lee YC. Relationship between unstimulated salivary flow rate and saliva composition of healty children in Taiwan. Chang Gung Med J 2008; 31: 281-286.
15) Guggenheimer J, Moore PA. Xerostomia: etiology, recognition and treatment. J Am Dent Assoc 2003; 134: 61-69.
16) Benderli Y, Erdilek D, Koray F, Telci A, Turan N. The relation between salivary IgA and caries in renal transplant patients. Oral Surg Oral Med Oral Pathol Oral Radio Endo 2000; 89: 588-593.
17) Cogulu D, Ertugrul S, Kutukculer N, Ozkinay F. Evaluation of the relationship between caries indices and salivary secretory IgA, salivary pH, buffering capacity and flow rate in children with

Down's syndrome. Arch Oral Biology 2006; 51: 23-28.

18) Barr-Agholme M, Dahllof G, Modeer T, Engstrom PE, Engstrom GN. Periodontal conditions and salivary and salivary immunoglobulins in individual with Down's syndrome. J Periodontol 1998; 69: 1119-1123.

19) Gavião MBD, Van der Bilt A. Chewing behavior and salivary secretion. J Appl Oral Sci 2004; 12: 159-163.

20) Kariyawasam AP, Dawes C. A circannual rhythm in unstimulated salivary flow rate when the ambient temperature varies by only about 2 degrees C. Arch Oral Biol 2005; 50: 919-922.

21) Yamamoto K, Kurihara M, Matsusue Y, Imanishi M, Tsuyuki M, Kirita T. Whole saliva flow rate and body profile in healthy young adults. Arch Oral Biol 2009; 54: 464-469.

22) Larsen MJ, Jensen AF, Madsen DM, Pearce EL. Individual variations of pH, buffer capacity, and concentrations of calcium and phosphate in unstimulated whole saliva. Arch Oral Biol 1999; 44: 111-117.

23) Rockembach MI, Marinho SA, Veeck EB, Linderman L, Shinkai RS. Salivary flow rate, pH, and concentrations of calcium, phosphate, and IgA in brazilian pregnant and non-pregnant women. Head Face Med 2006; 2: 44-48.

24) Moreira AR, Passos IA, Sampaio FC, Soares MSM, Oliveira RJ. Flow rate, pH and calcium concentration of saliva of children and adolescents with type 1 diabetes mellitus. Braz J Med Biol Res 2009; 42: 707-711.

25) Inoue H, Ono K, Masuda W, Morimoto Y, Tanaka T, Yokota M, Inegada K. Gender difference in unstimulated whole saliva flow rate and salivary gland sizes. Arch Oral Biol 2006; 51: 1055-1060.

26) Dawes C. Gland size estimation and body mass index improve salivary flow rate assessment. Arch Oral Biol 2007; 52: 409-410.

27) Nagler MR, Hershkovich O. Relationships between age, drugs, oral sensorial complaints and salivary profile. Arch Oral Biol 2005; 50: 7-16.

28) Richardson CT, Feldman M. Salivary response to food in humans and its effect on gastric acid secretion. Am J Physiol 1986; 250: 85-91.

29) Bokor-Bratic M, Cankovic M, Dragnic N. Unstimulated whole salivary flow rate and anxiolytics intake are independently associated with oral Candida infection in patients with oral lichen planus. Eur J Oral Sci 2013; 121: 427-433.

30) Torres RS, Peixoto BC, Caldas MD, Silva BE, Akiti T, Nucci M, De Uzeda M. Relationship between salivary flow rates and Candida counts in subjects with xerostomia. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002; 93: 149-154.